

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

1. (currently amended) A method for determining the amount of template nucleic acid present in a sample comprising ~~the steps of~~:

- i) bringing into association with the sample all the components necessary for nucleic acid amplification, and all the components necessary for a bioluminescence assay for nucleic acid amplification including:
 - a) a nucleic acid polymerase,
 - b) the substrates for the nucleic acid polymerase,
 - c) at least two primers,
 - d) a thermostable luciferase,
 - e) luciferin,
 - f) optionally ATP sulphurylase, and
 - g) optionally adenosine 5' phosphosulphate,

and subsequently:

- ii) performing a nucleic acid amplification reaction of the target nucleic acid involving more than one cycle of amplification;
- iii) monitoring the intensity of light output from the bioluminescence reaction, and
- iv) determining the amount of template nucleic acid present in the sample.

2. (currently amended) A method according to claim 1, wherein at least steps ii) and iii) are carried out in a sealed vessel.

3. (currently amended) A method according to claim 1, wherein in step iii) the intensity of light output is monitored during the nucleic acid amplification reaction.

4. (currently amended) A method according to claim 1, wherein step iii) further includes producing a data set of intensity of light output as a function of time.

5. (previously presented) A method according to claim 4, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of change of intensity of light output changes significantly.
6. (currently amended) A method according to claim 1 for determining the amount of template nucleic acid present in the sample at the beginning of the nucleic acid amplification reaction of step ii).
7. (currently amended) A method according to claim 1 for determining the amount of template nucleic acid present in the sample as a result of the nucleic acid amplification reaction of step ii).
8. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output begins to increase.
9. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output is at a maximum.
10. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of decrease of intensity of light output increases.
11. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of decrease of intensity of light output decreases.

12. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output reaches or crosses a predetermined level.

13. (currently amended) A method according to claim 8, wherein the thermostable luciferase that is brought into association with the sample in step i) is a reversibly-inhibited luciferase.

14. (currently amended) A method according to claim 1, wherein step iv) further comprises comparing the intensity of light output to the intensity of light output from a control in which the sample comprises a known amount of template nucleic acid.

15. (previously presented) A method according to claim 1 for determining whether the template nucleic acid is present in the sample.

16. (original) A method according to claim 14, wherein whether the template nucleic acid is present in the sample is determined by measuring from the data set whether the intensity of light output reaches or crosses a predetermined level.

17. (original) A method according to claim 15, wherein an increase in the intensity of light output relative to the predetermined level indicates the presence of template nucleic acid in the sample.

18. (original) A method according to claim 15, wherein a decrease in the intensity of light output relative to the predetermined level indicates the presence of template nucleic acid in the sample.

19. (currently amended) A method according to claim 16, wherein whether the template nucleic acid is present in the sample is determined by measuring from the data set

whether the intensity of light output reaches or crosses the predetermined level within a predetermined length of time following the start of the amplification reaction of ~~step~~ ii).

20. (currently amended) A method according to claim 1, wherein ~~step~~ iv) further comprises comparing the intensity of light output to the intensity of light output from a control in which no amplification has taken place.

21. (original) A method according to claim 20, wherein a decrease in the intensity of light output relative to a control reaction in which no amplification has taken place indicates the presence of template nucleic acid in the sample.

22. (currently amended) A method according to claim 1, wherein the nucleic acid amplification reaction of ~~step~~ ii) is a low temperature thermocycling amplification method in which the cycling temperature range does not exceed 75°C.

23. (currently amended) A method according to claim 1, wherein the nucleic acid amplification reaction of ~~step~~ ii) is carried out isothermally.

24. (currently amended) A method according to claim 23, wherein the nucleic acid amplification reaction of ~~step~~ ii) is carried out within a temperature range that does not exceed 75°C.

25. (currently amended) A method according to claim 23, wherein the nucleic acid amplification reaction of ~~step~~ ii) is carried out at a constant temperature at which the components of the amplification reaction and the bioluminescence assay are stable.

26. (currently amended) A method according to claim 23, wherein the nucleic acid amplification reaction of ~~step~~ ii) is carried out at more than one temperature within the temperature range in which the components of the amplification reaction and the bioluminescence assay are stable.

27. (currently amended) A method according to claim 26, wherein the nucleic acid amplification reaction of step ii) is started at a higher temperature and subsequently dropped to a lower temperature.
28. (previously presented) A method according to claim 1 for use in medical diagnostics.
29. (previously presented) A method according to claim 1 for use in determining whether a pathogen is present in a sample.
30. (previously presented) A method according to claim 1 for determining whether a particular nucleic acid sequence is present in an organism's genetic code.
31. (original) A method according to claim 30 for determining whether the nucleic acid to which the template nucleic acid originates has been genetically modified.
32. (previously presented) A method according to claim 1 for determining whether an organism is present in a sample.
33. (previously presented) A method according to claim 1 for use in immuno-nucleic acid amplification technology.
34. (currently amended) A kit for use in a method according to claim 1, wherein the kit comprises a nucleic acid polymerase, the substrates for the nucleic acid polymerase, at least two primers, a thermostable luciferase, luciferin, and optionally ATP sulphurylase, and optionally adenosine 5' phosphosulphate.
35. (previously presented) A kit for use in a method according to claim 1, wherein the kit comprises containers respectively containing:
- a) a buffered mixture of nucleic acid polymerase, a source of Mg and dNTPs; and
 - b) a luciferase, luciferin and ATP sulphurylase.

36. (previously presented) A kit according to claim 34, wherein at least one of the components of the kit is in a form which is suitable for storage in the kit.

37. (previously presented) A device for performing a method for determining the amount of template nucleic acid present in a sample, wherein said device incorporates the components that are present in a kit according to claim 34.